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Articles

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Abstract

Infectious diseases create major challenges for wildlife management. In particular, prion diseases are fatal and incurable, leaving managers with limited options. In cervids, chronic wasting disease (CWD) can decimate captive and wild populations by affecting neural tissue leading to body control loss, decay, and ultimately death resulting in ecological and economic consequences. Partial protection against CWD results from some genotypes at the prion (PRNP) locus encoding PrP proteins that are less likely to misfold and build up to fatal levels in the central nervous system. Although multiple studies have documented the association between CWD susceptibility and genotypes, little is known about the distribution of resistant genotypes across the natural landscape, and whether population pockets of protection in exist in particular regions. We surveyed the genetic variability and distribution of resistant alleles and genotypes of the PRNP locus across Nebraska in deer collected in 2017, where mule deer (*Odocoileus hemionus*) and white-tailed (*O. virginianus*) deer ranges meet on the North American Great Plains. We found that CWD-resistant alleles occur throughout the state in low frequencies, and our data suggest little evidence of geographic structure for the PRNP locus. In Nebraska, there is a lower frequency of the most common resistance allele (S96) compared with white-tailed deer in other parts of the Midwest. The frequency of resistant alleles (F225) was lower in mule deer. The low but widespread frequency of resistance alleles suggests that each species could be susceptible to CWD spread. Continued monitoring would be useful to determine if the frequency of resistant alleles increases in areas with increasing CWD rates. Three synonymous fixed genotypes at the PRNP locus allowed detection of hybrids between mule deer and white-tailed deer, although we found none, suggesting that CWD is not spread between species via hybridization. We also compare the PRNP genotypes of scrapie-resistant sheep with those of deer, and suggest that a single base-pair mutation at the PRNP locus could provide resistance in deer.

Keywords: disease resistance; genetic structure; mule deer; population management; sheep; white-tailed deer

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Introduction

Deer harvesting is an important activity with economic, cultural, and ecological impacts (Bishop 2004). Monitoring the health and spread of disease among wild deer populations is, thus, a major endeavor of game managers. Of particular recent interest to disease management is the spread of chronic wasting disease (CWD), a transmissible spongiform encephalopathy, which could potentially lead to extirpation of at least local populations of wild cervids (Almberg et al. 2011; Hayley and Hoover 2015). This currently incurable disease was first found in 1967 in wildlife research facilities in Colorado, formally described as CWD in 1978 from captive deer (genus *Odocoileus*) in Wyoming, recognized in free-ranging elk *Cervus elaphus* in Colorado in 1981, and in 1996 was found in elk farms in Saskatchewan, Canada, and in Nebraska, South Dakota, Colorado, Oklahoma, and Montana in the U.S. (Williams and Miller 2002). The disease in 2018 had spread to wild deer in 25 states in the United States, two Canadian provinces, the Republic of Korea, Norway and Finland, and has expanded to moose *Alces alces* and reindeer *Rangifer tarandus* (CDC 2019), causing many game agencies to implement programs to limit and track spread (Frost et al. 2009; Plummer et al. 2018).

Chronic wasting disease is thought to be transmitted through direct horizontal transfer between individuals in contact with urine, feces, or saliva (Miller et al. 2000), indirectly by consuming prions deposited in the environment (Miller et al. 2004; Mathiason et al. 2009; Gough and Maddison 2010), by contact with other deer (VerCauteren et al. 2007), and possibly via rodents (Heisey et al. 2010). Environmental transmission is of special concern because infectious prions can remain in water and soils for long after an infected animal has contaminated the site (Nichols et al. 2009; Plummer et al. 2018), increasing the probability of disease spread via an ever-increasing reservoir of prions. In the relatively early stages of CWD, there have been different disease trajectories in different areas. In Colorado, the first known area with a major outbreak, some areas have witnessed >40% infection rates in males, whereas in other areas infection rates have remained lower (overall in 2015–2017, 15% of Colorado white-tailed deer *Odocoileus virginianus* tested positive for CWD). In Wisconsin, where an ongoing epidemic is occurring west of Madison, increasingly large percentages of adult males and females exhibit CWD symptoms. Whatever the ultimate cause of the emergence of CWD in wild cervids (see Bastian 2014, 2017), management efforts to limit spread often involve culling (Wasserberg et al. 2009; Jennelle et al. 2014), along with recommendations against supplemental feeding, mineral licks (Plummer et al. 2018), or any human activity that concentrates deer. Storm et al. (2013) and Jennelle et al. (2014) suggest that the mode of transmission is not density-dependent, which makes disease control via culling difficult once the disease has become common in an area.

One potential factor that might reduce the rate and extent of spread of CWD involves resistance to CWD provided by certain genotypes at the prion protein (PrP) encoded by the PRNP locus (Robinson et al. 2012b). Partially resistant genotypes in white-tailed deer occur at PrP amino acid positions 95 and 96, where glutamine (Q) is replaced by histidine (H) and glycine (G) is replaced by a serine (S), respectively, and in mule deer *O. hemionus,* where serine (S) is replaced by phenylalanine (F) at position 225 (O'Rourke et al. 1998, 2004; Raymond et al. 2000; Heaton et al. 2003; Johnson et al. 2003; Kelly et al. 2008). The wild type, or most common, allele in white-tailed deer is therefore denoted as Q95G96A116S225. Johnson et al. (2011) and Race et al. (2011) showed experimentally that white-tailed deer orally inoculated with prions from CWD-positive deer lived for different lengths of time depending on their PRNP genotype. In particular, individuals having the H95 allele had the longest incubation period, surviving for >1,500 days postinoculation, whereas individuals with the S96 survive around 1,000 days. In contrast wild-type Q95 and G96 individuals did not survive past 550 days. O'Rourke et al. (2004), Edmunds et al. (2016) and Brandt et al. (2015, 2018) reported similar results. However, sample sizes were of necessity relatively small, and it is not clear that deer could obtain the same high dosage of prions in the wild as were used in experiments. For example, Johnson et al. (2011) remarked that, in contrast to their experimental procedure, "exposure to CWD agent would likely be sporadic and at a substantially lower dose" in nature. The wild-type allele in mule deer is also Q95G96A116S225. In mule deer, Jewell et al. (2005) found that individuals with the 225SS were 30 times more likely to be CWD-positive than those with 225SF. We note that the studies mentioned above found that all deer succumbed to CWD; hence, resistance is apparently not absolute, and all deer irrespective of their genotype are probably susceptible to CWD. Hence, unlike sheep in which a particular genotype confers resistance to scrapie, complete resistance appears not to exist at this time in white-tailed deer or mule deer.

The existence of genotypes that provide partial resistance or extend the incubation period of CWD could have management implications. If, for example, there were areas in which most deer carried resistant alleles, it might be less important to monitor and manage them owing to a reduced level of overall susceptibility. Therefore, as a case study, we documented the geographic distribution and frequency of genotypes at the PRNP locus of wild hunter-harvested mule deer and white-tailed deer in Nebraska. To date, detailed geographic distribution of resistant alleles across a landscape at the state-level management scale is unknown. Being centrally located in the Great Plains where both *Odocoileus* species meet (Jones 1964), Nebraska offers a useful system to study the geographic distribution of CWD-resistant alleles (Brandt et al. 2018). Mule deer are more common in the west; they are gradually replaced by white-tailed deer to the east, although both are found in much of Nebraska segregated by habitat (Bailey et al. 1957; Jones 1964; Whitehead

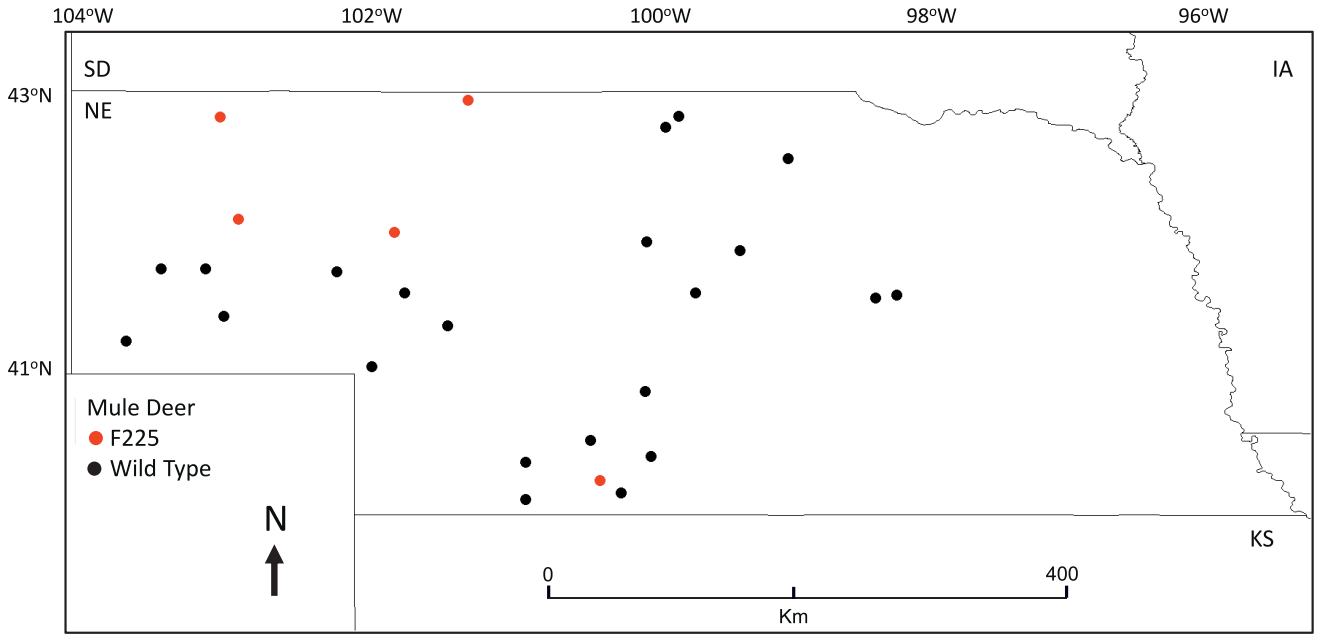

Figure 1. Distribution of samples (circles) of mule deer *Odocoileus hemionus* in Nebraska taken in 2017 showing sparse representation of the partially resistant allele (red circles); frequency of F225 does not exceed 5% in any of the five samples where the allele was detected (see Data S1, *Supplemental Material*).

1972). Previous studies have concentrated on particular regions with high CWD incidence in a given state or province (e.g., Wisconsin, Illinois, Nebraska, Alberta) or have lacked geographic extent. Our goal was to determine if some geographic areas had greater frequencies of resistant genotypes in Nebraska. Lastly, because mule deer and white-tailed deer hybridize (e.g., Gutiérrez et al. 2017), we determine whether any individuals we examined were of hybrid origin, which could promote interspecies spread of CWD.

Methods

Using samples of free-ranging deer harvested by Nebraska Game and Parks personnel and private hunters in Nebraska during seasons 2016 and 2017, we extracted DNA using a DNeasy kit (Qiagen, Germantown, MD following the manufacturer's instructions. We amplified the open reading frame (ORF) on exon 3 of the PRNP locus. We set polymerase chain reactions in a 25-μL volume with Green GoTaq master mix (Promega, Fitchburg, WI) and checked them in 1% agarose gels stained with GelRed (Biotium, Fremont, CA). Universal PRNP primers can coamplify a processed pseudogene in some individuals and overestimate polymorphism levels (Brayton et al. 2004; O'Rourke et al. 2004). Thus, we amplified the PRNP locus using functional copy-specific primers that bind to intron 2 (forward primer 223: 5′-acaccctctttattttgcag-3′, and reverse primer 224: 5′-agaagataatgaaaacaggaag-3′; O'Rourke et al. 2004). Polymerase chain reaction thermocycling conditions (O'Rourke et al. 2004) were as follows: initial denaturing 95°C for 5 min, followed by 30 cycles of denaturation (95°C, 30 s), annealing (54°C, 30 s) and extension (72°C, 59 s), followed by a final extension (72°C, 7 min). Genewiz (South Plainfield, NJ) purified and Sanger-sequenced polymerase chain reaction products by using ExoSap-it and an ABI Prism 3730xl DNA analyzer, respectively. We edited chromatograms in SEQUENCHER 5.4 (Gene Codes, Ann Arbor, MI). Finally, we separated alleles bearing heterozygote sites using in silico phasing in PHASE 2.1 (Stephens et al. 2001) by interconverting the original aligned FASTA files in SEQPHASE (Flot 2010), running 100,000 generations with a burn-in of 10,000 using a 0.7 posterior probability. We then translated nucleotide sequences to amino acids. Using the methods described above, we also genotyped a sample of 15 white-tailed deer from Minnesota to extend our geographic comparison to another state with no data on frequencies of resistance alleles. None of the deer used in this study were tested for CWD. We scored allele resistance based on extensive evidence that particular genotypes have been associated with CWD prevalence by multiple studies (see Haley and Hoover 2015). All PRNP sequences from this study are deposited in GenBank (MN 389787–390610).

To test whether genetic variation at the PRNP locus is geographically structured, we grouped individuals sampled within a 10-mile radius (17 km) into 28 geographic samples of mule deer and 53 samples of white-tailed deer (Figures 1, 2). Although this resulted in a mean of 5 deer per geographic sample, we note that because the locus is diploid, we sampled 10 alleles on average per sample (Data S1, *Supplemental Material*). To provide a check on geographic structuring that might be an artifact of these relatively small samples, we also grouped white-tailed deer into larger geographic groups (west [$n = 120$], central [168], east [195]) and mule deer

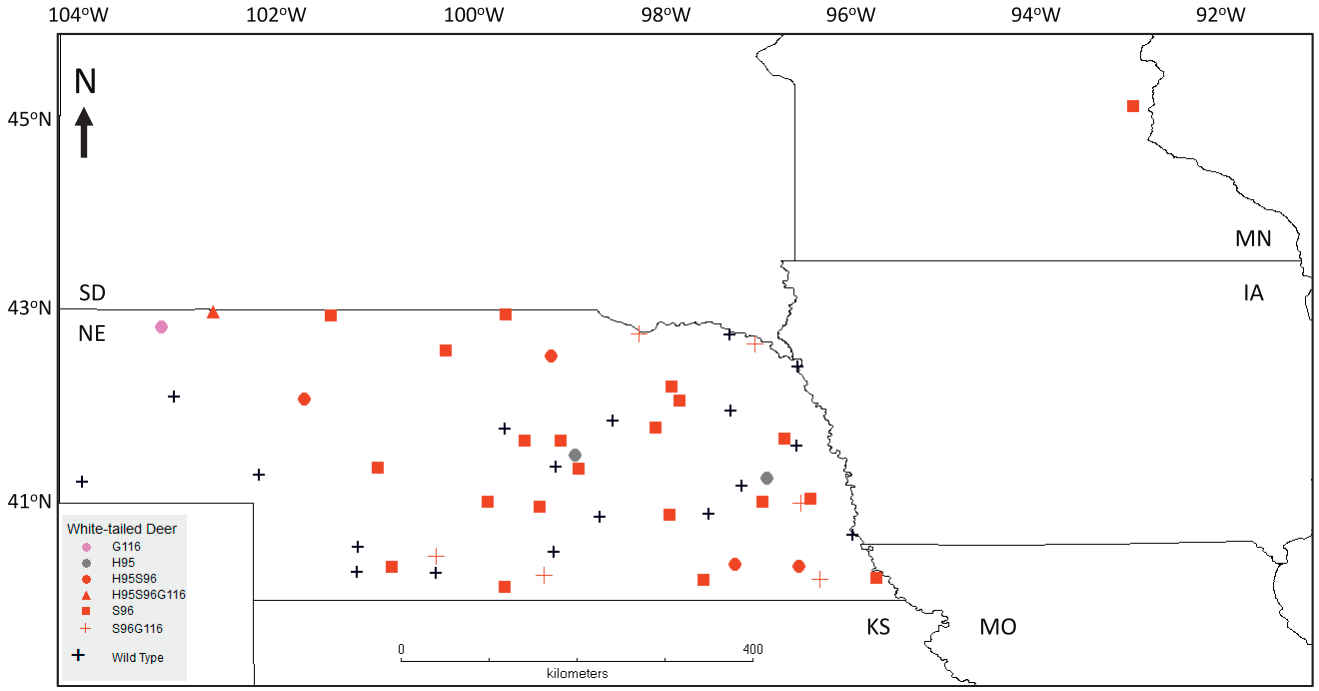

Figure 2. Distribution of samples (various symbols) of white-tailed deer *Odocoileus virginianus* and presence of the partially resistant alleles for white-tailed deer in Nebraska taken in 2017 (see legend). The frequency of each resistance allele (e.g., H95, S96, G116) in each sample is given in Data S1 (*Supplemental Material*), but in all cases is low.

into two groups (west [$n = 92$], east [177]). To explore the nature of genetic variation and differentiation, we computed nucleotide and allele diversities, and a fixation index statistic (G_{ST}) for each species in DnaSP5 (Librado and Rozas 2009). We tested for isolation by distance using a Mantel test of linearized $G_{ST}/1 - G_{ST}$ regressed over log-transformed geographic distances in km (Rousset 1997). We compared gene sequences from the two species to determine whether fixed differences indicated the presence of hybrid individuals. As part of a different study, we sequenced 959 base pairs of the mitochondrial cytochrome *b* (Cyt *b*) gene for mule deer ($n = 66$) and white-tailed deer ($n = 102$) in Nebraska. We grouped these sequences into the same three large geographic groupings as those used for PRNP and report here briefly the degree of geographic structure at this locus to complement the PRNP data.

We tested whether nonsynonymous amino acid replacements had a significant functional change in the prion using the software Protein Variation Effect Analyzer (PROVEAN; Choi et al. 2012). Briefly, PROVEAN uses a reference sequence (wild-type allele) and compares amino acid substitutions with a curated database of protein sequences (The Uniprot Consortium 2019), aligns the reference protein to close matches as judged by Basic Local Alignment Search Tool E-values, forms clusters based on similarity, and then calculates distances within and among protein sequence clusters, averaging them for a final score. If the score is larger than a −2.5 threshold, amino acid replacements are considered neutral. If score values are equal to or smaller than −2.5 they are considered deleterious. Lastly, many interpretations of the origin of CWD in deer assume that the initial infection came from copenned sheep and mule deer in Colorado (Spraker et al. 1997). Sheep possess a genotype resistant to scrapie; therefore, we compared the base pair composition at amino acid sites that influence resistance in sheep and deer, to ascertain what genetic changes would have to evolve at the PRNP locus to result in deer exhibiting the scrapie-resistant genotype observed in sheep.

Results

We genotyped 137 mule deer (8 females) and 264 white-tailed deer (58 females) for the exon 3 ORF of the PRNP locus (Data S1). Our DNA sequencing produced 771 base pairs (bps) that, when translated into amino acids, correspond to 256 positions and one final stop codon. We found no stop codons elsewhere in our sequences. We used functional-specific primers, so we did not detect the S138N mutation; this indicates that the genetic variability we found is not due to coamplified pseudogenes (Brayton et al. 2004; O'Rourke et al. 2004).

For mule deer, the average number of segregating sites was 1.4, haplotype number was 2.46, haplotype diversity was 0.52, nucleotide diversity (π) was 0.00081 (Data S1), frequency of the wild-type allele was 97.1% (Table 1), and the resistant allele (F225) was uncommon and more often encountered in the northwestern part of the state (Figure 1). Another PRNP nonsynonymous substitution in mule deer (D20G) exists (Brayton et al. 2004; Wilson et al. 2009), but there is no definitive evidence of it conferring CWD protection (Brayton et al. 2004; Jewell et al. 2005; Haley and Hoover 2015). We

Table 1. Genotypic and allelic frequencies of the functional PRNP gene in Nebraska mule deer *Odocoileus hemionus* and white-tailed deer *O. virginianus* taken in 2017. Wild-type alleles correspond to a QGAS genotype at amino acid positions 95, 96, 116, and 225, respectively, on the open reading frame of exon 3 on the PRNP locus. Mutant alleles at H95, S96, G116, and F225 are considered to confer partial resistance to chronic wasting disease (O'Rourke et al. 2004; Jewell et al. 2005; Johnson et al. 2011; Robinson et al. 2012b).

Genotypes	Individuals	Alleles[a]	Percent
Mule deer			
QGAS QGAS	130–94.9%	QGAS	97.1
QGAS QGAF	6–4.3%	QGAF	2.9
QGAF QGAF	1–0.7%	—	—
Total	137	—	—
White-tailed deer			
QGAS QGAS	162–65.1% (+8 MN)	QGAS	79.7
QGAS QSAS	58–23.3% (+5 MN)	QSAS	16.7
QSAS QSAS	12–4.8% (+2 MN)	QGGS	2.0
QGAS QGGS	9–3.6%	HGAS	1.6
QGGS QGGS	0%	—	—
QGAS HGAS	6–2.4%	—	—
HGAS HGAS	1–0.4%	—	—
QSAS QGGS	1–0.4%	—	—
QSAS HGAS	0%	—	—
QGGS HGAS	0%	—	—
Total	249 (+15 MN)	—	—

[a] Allelic frequencies computed using O'Rourke et al. (2004) equation: [(Homozygote individuals × 2) + (Heterozygote individuals)] / (Total individuals × 2).

found G20 at a 10.9% frequency occurring in heterozygous state in all but one animal, and we found no individuals carrying G20 in combination with F225; we list its occurrence in the event it is later shown to influence susceptibility (see also Table 2; Data S1). The fixation index G_{ST} value was 0.0131 in mule deer and genetic differentiation estimates suggested no significant geographic differences among samples at the PRNP locus ($\chi^2 = 119.17$, $P = 0.83$, $df = 135$). The Cyt *b* data also exhibited no significant differences between the two large groups ($G_{ST} = -0.01$, $\chi^2 = 9.40$, $df = 12$, $P = 0.668$).

For the PRNP locus in white-tailed deer, the average number of segregating sites was 3.6, number of haplotypes was 4.25, haplotype diversity was 0.79, nucleotide diversity was 0.00172 (Data S1), frequency of the wild-type allele was 79.7%, and the resistant alleles (H95, S96, and G116) were also relatively widespread but uncommon (Figure 2). The G_{ST} value was 0.013 (χ^2 =

Table 2. PRNP locus polymorphisms, synonymous (SYN) and nonsynonymous (NON-SYN) substitutions Nebraska mule (OH [*Odocoileus hemionus*]) and white-tailed (OV [*Odocoileus virginianus*]) deer taken in 2017. Mutation order, species (spp), amino acid position (pos), codon, amino acid replacement (AAsubs), equivalent nucleotide sequence place and the third codon position (bps), PROVEAN test of functional mutation change, and its prediction (–2.5 or smaller for deleterious effects; Choi et al. 2012).

Mutation	Spp	Pos	Codon	AAsubs	bps	PROVEAN	Prediction
NON-SYN							
1	OH	20	GAC/GGC	D>G	60	−0.928	NEUTRAL
2	OV	95	CAA/CAT	Q>H	285	−1.753	NEUTRAL
3	OV	96	GGT/AGT	G>S	288	−1.25	NEUTRAL
4	OV[a]	103	AAC/ATC	N>I	309	−2.729	DELETERIOUS
5	OV	116	GCA/GGA	A>G	348	−0.747	NEUTRAL
6	OV[a]	123	GCA/ACA	A>T	369	−1.418	NEUTRAL
7	PSEUDO	138	S>N	414	−0.517	NEUTRAL	
8	OH	225	TCC/TTC	S>F	675	−1.503	NEUTRAL
9	OV	226	CAG/AAG	Q>K	678	−0.511	NEUTRAL
Mutation	**Spp**	**Pos**	**CODON**	**AA**	**bps**		
SYN							
1	OV	51	CGC/CGT	R	153		
2	OV	81	GGT/GGA	G	243		
3	OV	108	CAA/CCG	P	324		
4	OH	131	TAT/TAC	Y	393		
5	OV	146	AAC/AAT	D	438		
6	OV	185	ATT/ATC	I	555		
7	OH[a]	238	TTC/TTT	F	714		
8	OH	247	ATC/ATT	I	741		

[a] Novel mutations.

1,360.45, $P = 0.00001$, $df = 1{,}020$), suggesting some heterogeneity across the landscape in white-tailed deer. However, for the three larger groupings, the G_{ST} values were all <0.01 and nonsignificant. We found three nonsynonymous fixed differences between mule deer and white-tailed deer (positions 417, 468, 606), and no individuals were heterozygous, suggesting at most a low frequency of hybridization between mule deer and white-tailed deer in Nebraska. The Cyt *b* data divided into the three broad groups revealed no geographic structure across the state ($G_{ST} = 0.001$, $\chi^2 = 33.65$, $df = 30$, $P = 0.295$). At the PRNP locus we found no evidence of isolation by distance in mule deer ($R^2 = 0.001$, $P = 0.46$), and a weak yet significant effect of isolation by distance in white-tailed deer ($R^2 = 0.003$, $P = 0.02$). Cyt *b* data are deposited in Genbank (MN390611–390813).

We found all previously reported nonsynonymous and synonymous amino acid substitutions for mule and white tailed deer, and discovered variants not previously reported in the literature (Table 2; Robinson et al. 2012b). We detected novel nonsynonymous substitutions in white-tailed deer at amino acid position 103, where asparagine was replaced by isoleucine (N103I), and at amino acid position 123, where alanine was replaced by threonine (A123T). According to PROVEAN results, mutation predictions in most cases were greater than −2.5 (ranging −0.51 to −1.75) and thus considered neutral (i.e., not changing protein function). The novel amino acid substitution N103I had a PROVEAN score of −2.79, suggesting a deleterious change (i.e., significant change in protein function). The frequency of the I103 substitution was 0.9% of white tailed deer (five heterozygote individuals) from Harlan, Johnson, Lincoln, Wheeler, and York counties (CWD has been detected in Harlan and Lincoln counties). In mule deer we detected one novel synonymous mutation at nucleotide position 714, corresponding to phenylalanine on amino acid position 238 (F238). For PRNP allele frequencies, we found a deficit of the most common resistant allele S96 in free-ranging white-tailed deer ($\chi^2 = 22.028$, $df = 3$, $P = 6.435 \times 10^{-5}$). Similarly, we found significantly fewer resistant genotype combinations for six nonzero genotypic frequencies in free-ranging white-tailed deer ($\chi^2 = 84.274$, $df = 5$, $P < 2.2 \times 10^{-16}$; Table 1).

Discussion

The spatial distribution of animals carrying genetic resistant to diseases could influence how wildlife populations are managed. At the PRNP locus, the amount of genetic variance distributed among localities for both species (G_{ST}) was <2%, suggesting low genetic heterogeneity; that is, there were no geographic samples with high frequencies of H95 or F225 resistance-type alleles (Data S1). The significant G_{ST} value at the PRNP locus for the 53 individual geographic samples of white-tailed deer could be an example of statistical and not biological significance (Hedrick 2001; Nakagawa and Cuthill 2007), given that the G_{ST} value for the three pooled samples was insignificant. The relatively greater genetic diversity at PRNP in white-tailed deer is likely a result of a greater population density in Nebraska, and the existence of more than one alternative allele. In accordance with the PRNP data, the Cyt *b* sequences showed no significant geographic genetic structure. The absence of strong geographic patterns of genetic variation at the PRNP and Cyt *b* loci in each deer species suggests relatively few barriers to gene flow. If a lack of structure is confirmed with additional loci, both deer species could be considered equally susceptible to CWD throughout the state. We note that a larger sample of loci is needed to yield strong inferences about overall genetic population structure.

Areas with longer exposure times to CWD might have different frequencies of resistance alleles because of selection against wild-type alleles (Robinson et al. 2012a; Miller and Walter 2019). Variation in the frequencies of resistance alleles across the range of white-tailed deer has been documented both in areas with and without CWD (Johnson et al. 2006; Kelly et al. 2008; Wilson et al. 2009; Brandt et al. 2015, 2018; Haley and Hoover 2015). For example, results from CWD-endemic areas such as Wisconsin and Illinois exhibit a higher frequency of the S96 allele in wild populations (~40%; Johnson et al. 2006; Kelly et al. 2008) than we found in Nebraska. Areas in Illinois where CWD was recently detected tend to have a higher frequency of resistant alleles compared with places with a longer CWD history (Brandt et al. 2018). In the sample from a CWD-free zone in Washington County, Minnesota, we estimated that 30% of individuals have the S96 allele (Table 1). In Nebraska, testing since 1997 has revealed fewer than 1% CWD-positive deer; however, in 2017, 1,807 deer (including both species mostly from western Nebraska) revealed 203 positives (11.2%; Kane 2018), an indication of increasing frequency. It will be useful to monitor areas with emergent CWD outbreaks such as Nebraska to determine if frequencies of resistant alleles increase over time.

Having a range-wide survey similar to the one we present here for Nebraska could help clarify these geographic frequency patterns, and provide for a proactive approach to CWD management in the 25 U.S. states currently lacking the disease (CDC 2019). For example, the endangered (US Endangered Species Act [ESA 1973] as amended) Key deer *O. v. clavium* have a high frequency of the S96 allele, whereas wild-type alleles are far more frequent in Coues deer *O. v. couesi* in Arizona and black-tailed *O. h. sitkensis* and *O. h. columbianus* deer from the Pacific Northwest, respectively (H.V.M. and R.M.Z., unpublished data). An issue that prevented us from making a broader analysis is that thousands of sequences used in PRNP genotyping studies, except those from Brandt et al. (2018) and those we report here, are not publically available in appropriate repositories (e.g., Genbank, The European Molecular Biology Open Software Suite). We encourage other researchers to release their sequences and corresponding geographic information, which would greatly aid studies of CWD genetic resistance.

Compared with white-tailed deer, mule deer have fewer known CWD-resistant alleles, and the ones they do possess are in low frequency. Our data from Nebraska are

comparable to frequencies from management units in Wyoming ranging between 0% and 8% (Jewell et al. 2005), albeit on the lower end of the spectrum. In Canada, F225 was not found in >200 animals (Wilson et al. 2009). At the other extreme, in the CWD-endemic area of Colorado, the F225 frequency was 18.1% (Jewell et al. 2005). This wide range of resistance frequency in mule deer mimics the values seen in white-tailed deer and correlates with a higher frequency of resistant alleles from CWD-endemic areas. It is likely that removal of individuals with wild-type alleles either by CWD mortality or by culling in outbreak areas resulted in an increased frequency of resistant alleles. However, the low frequency of F225 suggests that mule deer in Nebraska have not as yet experienced selection for greater resistance in response to CWD outbreaks in the same way as white-tailed deer. The absence of F1 hybrids between mule deer and white-tailed deer in our samples suggests that hybridization is not major route for CWD spread between species, although in some areas, hybrids make up >5% of the population (Bradley et al. 2003).

Studies aimed at assessing the relationship between PRNP genotypes and CWD likely stem from the observation that a particular genotype in sheep confers immunity to scrapie (Fernández-Borges et al. 2018). Thus, we explored the genetic changes that would be required for deer to evolve the level of resistance observed in sheep. In a survey of PRNP sequences on Genbank, we found 3 fixed amino acids and 19 fixed base-pair differences on average between wild-type sheep and deer. In sheep, the positions relevant to scrapie resistance are 136 (A/V), 154 (R/H), and 171 (Q/R/H), with the 136A154R171R genotype conferring complete or nearly complete resistance (Hagenaars et al. 2018). At the seven amino acid positions implicated in CWD resistance, sheep and deer are often the same: Q(95), G(96), A(116), A(136), R(154), Q(171), Q(226). Hence, deer might obtain the resistance seen in sheep with a change at position 171 from Q to R; at the DNA level, from CAG to CGG. The PROVEAN score for such a change, −0.899, is considered neutral. For comparison, all substitutions in sheep to ARR are also neutral. In a survey of >50 mammalian species (R.M.Z., unpublished data), Q is the common amino acid at position 171, suggesting that the existence of an R is a result of an infrequent mutation. Nonetheless, it would appear that deer only require a transition at a second base position to acquire resistance, although none of the individuals we sequenced possessed that base substitution. It might also be the case that resistance to CWD might require different amino acid sequences than those involved in resistance to scrapie.

The mechanism by which different genotypes affect CWD expression is not perfectly clear, but Velásquez et al. (2015) suggest that different allelic combinations yield novel CWD strains that might be differentially susceptible to misfolding (Hannaoui et al. 2017). To our knowledge, there are no published estimates of the potential biological effects of nonsynonymous mutations in prion variability associated with CWD. Except for one, all amino acid substitutions were functionally neutral (Table 2), which one might expect because resistant alleles would not be expected to be deleterious, as noted above for sheep. In contrast, the novel N103I substitution in white-tailed deer has significant effects on protein biological function, likely from the replacement of a polar side chain amino acid to a nonpolar side chain (Betts and Russell 2003). The frequency of I103 in Nebraska was quite low (<1.0%), and its rarity is probably a result of its deleterious effects.

An obvious question is whether having individuals with resistant alleles will have a population effect in the face of a CWD outbreak (Brandt et al. 2018). In sheep, breeding only scrapie-resistant animals has been implemented successfully (Drögemtuller et al. 2001; Melchior et al. 2010; Haley and Hoover 2015). In wild deer populations, having greater prevalence of resistant alleles might be a double-edged sword. On the one hand, more resistant animals would live longer and have greater lifetime reproductive success, thus mitigating CWD-caused population declines. On the other hand, infected deer with resistant alleles will carry the disease for longer, potentially increasing the probability of CWD spread, unless resistant genotypes are less likely to shed prions. An extended lifespan is particularly worrisome because there is evidence of animals shedding infective prions in urine and feces before CWD symptoms appear (Plummer et al. 2017). However, it is unknown when during the course of an infection individuals with resistant genotypes begin shedding sufficient numbers of prions to be infectious to other deer. In other words, when is an infected deer an immediate direct threat to other deer—when it has a single prion, 10 prions, 1,000 prions, or some greater level? If in the future, reintroductions of deer are used to counter population declines due to CWD, it could be valuable to release deer with less susceptible genotypes.

Management Implications

The alleles associated with partial resistance and longer incubation periods to CWD in both white-tailed deer and mule deer are widespread throughout the landscape but in low frequency across Nebraska. The DNA data suggest very low genetic differentiation among geographic samples at two loci, meaning that gene flow potentially connects the deer herd throughout the state. Ours is the first attempt we are aware of to provide a fine-scaled picture of the geographic distributions of alleles affecting CWD over a relatively broad area. In our opinion, the low frequency and widespread distribution of resistance alleles suggests that the entire Nebraska herd should be considered equally genetically susceptible to a CWD outbreak. That is, we found no pockets of resistance that might serve as refugia for deer in the case of an outbreak.

Supplemental Material

Please note: The *Journal of Fish and Wildlife Management* is not responsible for the content or functionality of any

supplemental material. Queries should be directed to the corresponding author for the article.

Data S1. Locations and genetic characteristics (numbers and frequencies of resistant alleles, nucleotide diversity, haplotype diversity) of each sample of mule deer and white-tailed deer.

Found at DOI: https://doi.org/10.3996/012019-JFWM-002.S1 (67 KB XLS).

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